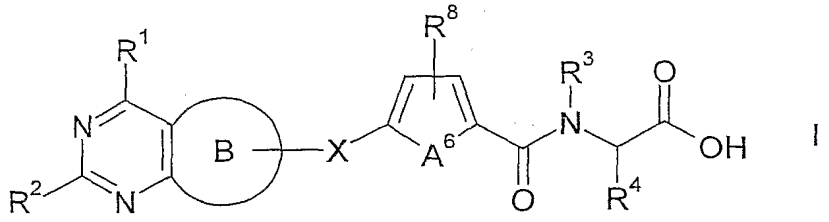
## CLAIMS

1. A method of combating toxicity caused by an antifolate compound of Formula I,



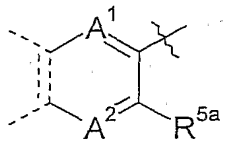
I

wherein

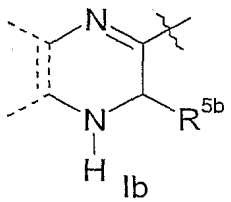
$R^1$ represents $NH_2$, OH or $CH_3$;

$R^2$ represents $NH_2$ or $C_{1-4}$ alkyl;

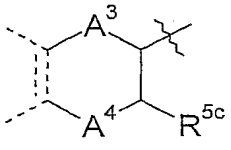
the group B represents a structural fragment of Formula Ia, Ib, Ic, Id or Ie,



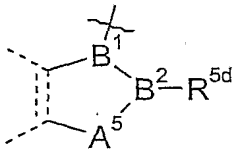
Ia



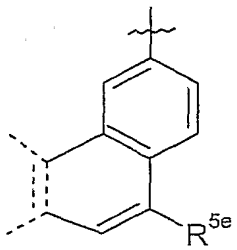
Ib



Ic



Id



Ie

in which groups the dashed lines indicate the point of ring fusion with the pyrimidinyl ring and the wavy lines indicate the point of attachment of the structural fragments to the group X;

$R^{5a}$ to $R^{5e}$ independently represent H or $C_{1-4}$ alkyl;

$A^1$ represents $C(R^{6a})$ or N;

$A^2$ represents CH or N;

$A^3$ represents $C(H)R^{6b}$, $NR^{6c}$ or S;

$A^4$ and $A^5$ independently represent $CH_2$, NH, O or S;

the group $B^1$-$B^2$ represents CH-CH or C=C;

$R^{6a}$ to $R^{6c}$ independently represent H or $C_{1-4}$ alkyl, or $R^{6c}$ represents $C(O)R^{6d}$, or $R^{6c}$, together with $R^{7b}$ represents $C_{1-2}$ *n*-alkylene;

$R^{6d}$ represents H or $C_{1-4}$ alkyl;

X represents $-CH_2C(H)R^{7a}-$ or $-CH_2NR^{7b}-$ (in which latter two groups the $CH_2$ moiety is attached to the fused, pyrimidine-based heterocyclic group);

$R^{7a}$ and $R^{7b}$ independently represent H, $C_{1-6}$ alkyl, $C_{3-6}$ alkenyl or $C_{3-6}$ alkynyl, or $R^{7b}$, together with $R^{6c}$ represents $C_{1-2}$ *n*-alkylene;

$A^6$ represents O or S;

$R^8$ represents H or one or two substituents selected from halo, $C_{1-4}$ alkyl and $C_{1-4}$ alkoxy;

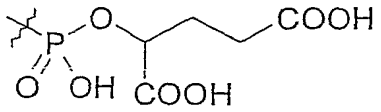
$R^3$ represents H or $C_{1-4}$ alkyl;

$R^4$ represents $-CH_2C(R^{9a})(R^{9b})$-D;

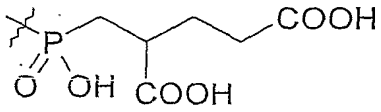
$R^{9a}$ and $R^{9b}$ independently represent H or $C_{1-4}$ alkyl, or $R^{9a}$ and $R^{9b}$ together represent $=C(H)R^{10}$;

$R^{10}$ represents H or $C_{1-4}$ alkyl;

D represents C(O)OH, tetrazol-5-yl, $(CH_2)_{0-1}$-$NHR^{11}$, or, when $R^{9a}$ and $R^{9b}$ together represent $=C(H)R^{10}$, then D may also represent H, or D represents a structural fragment of Formula IIIa or IIIb,



IIIa



IIIb ;

wherein the wavy lines indicate the point of attachment of the structural fragments;

$R^{11}$ represents H or $C(O)R^{12}$;

$R^{12}$ represents H or phenyl substituted by C(O)OH and optionally substituted by one or two further substituents selected from halo, $C_{1-4}$ alkyl and $C_{1-4}$ alkoxy; and

alkyl, alkenyl and alkynyl groups, as well as the alkyl part of alkoxy groups, may be substituted by one or more halo atoms;

or a pharmaceutically acceptable salt and/or solvate thereof,

in an individual who has been administered said compound, the method comprising administering to the individual an enzyme that has carboxypeptidase G activity.

2. A method according to Claim 1 wherein the antifolate compound of Formula I is Tomudex (Formula IV), LY309887 (Formula V), AG2034 (Formula VI) or AG2037 (Formula VII).

3. A method according to Claim 1 or 2 wherein the individual is administered the enzyme that has carboxypeptidase G activity between about 24 and 48 hours after being administered the antifolate compound.

4. A method according to any of Claims 1 to 3 wherein the individual has one of more clinical markers of toxicity caused by the antifolate compound.

5. A method according to Claim 4 wherein the clinical marker of toxicity caused by the antifolate compound is a plasma level of the compound greater than a predetermined level indicating toxicity at a given time after administration of the compound.

6. A method according to Claim 5 wherein the predetermined blood plasma level of the antifolate compound indicating toxicity is 1μM at 24 hours after administration of the compound.

7. A method according to Claim 5 or 6 further comprising the prior step of determining the plasma level of the antifolate compound in the individual at a given time after administration of the compound.

8. A method according to any of Claims 1 to 7 wherein the individual has one or more clinical symptoms of toxicity caused by the antifolate compound.

9. A method according to Claim 8 wherein the clinical symptom of toxicity caused by the antifolate compound is selected from anaemia, anorexia, asthenia, dehydration, diarrhoea, fatigue, fever, hepatotoxicity, hyperbilirubinaemia, leukopaenia, mucositis, myelosuppression, nausea, neutropaenia, rash, reversible transaminitis, stomatitis, thrombocytopaenia and vomiting.

10. A method according to Claim 8 or 9 further comprising the prior step of determining the presence of the one or more clinical symptoms of toxicity caused by the antifolate compound in the individual.

11. A method according to any of Claims 1 to 10 and further comprising administering a folate pathway rescue agent to the individual.

12. A method according to Claim 11 wherein the antifolate compound of Formula I is an inhibitor of dihydrofolate reductase (DHFR) or of glycinamide ribonucleotide formyltransferase (GARFT), and the folate pathway rescue agent is leucovorin calcium.

13. A method according to Claim 12 wherein the antifolate compound is LY309887, AG2034, or AG2037.

14. A method according to Claim 11 wherein the antifolate compound of Formula I is an inhibitor of thymidylate synthase (TS), and the folate pathway rescue agent is thymidine.

15. A method according to Claim 14 wherein the antifolate compound is Tomudex.

16. A method according to any of Claims 11 to 15 wherein the individual is administered the enzyme that has carboxypeptidase G activity prior to the folate pathway rescue agent.

17. A method according to any of Claims 11 to 15 wherein the individual is administered the folate pathway rescue agent prior to the enzyme that has carboxypeptidase G activity.

18. A method according to any of Claims 11 to 15 wherein the individual is administered the folate pathway rescue agent and the enzyme that has carboxypeptidase G activity substantially simultaneously.

19. A method according to any of Claims 1 to 18 wherein the individual is administered the enzyme that has carboxypeptidase G activity at a dose of about 50 Units per kg body weight.

20. A method of treating a disease selected from cancer, rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis, extrauterine pregnancy and graft vs. host disease comprising administering an antifolate compound of Formula I to the individual, and subsequently administering to the individual an enzyme that has carboxypeptidase G activity.

21. A method according to Claim 20 wherein the antifolate compound of Formula I is Tomudex and the cancer to be treated is cancer of the breast, ovary,

colon/rectum, liver, prostate, pancreas or stomach, or non small cell lung cancer (NSCLC), malignant mesothelioma or carcinoma of unknown primary.

22. A method according to Claim 20 wherein the antifolate compound is LY309887 and the cancer to be treated is cancer of the breast, colon, lung or pancreas.

23. A method according to Claim 20 wherein the antifolate compound is AG2034 and the cancer to be treated is breast cancer, colon cancer, lung cancer, melanoma or lymphoma.

24. A method according to Claim 20 wherein the antifolate compound is AG2037 and the cancer to be treated is a solid tumour, such as an advanced, metastatic or recurrent solid tumour.

25. Use of an enzyme that has carboxypeptidase G activity in the preparation of a medicament for combating toxicity caused by an antifolate compound of Formula I as defined in Claim 1 or Claim 2.

26. Use according to Claim 25 for combating toxicity in an individual who has one of more clinical markers of toxicity caused by the antifolate compound.

27. Use according to Claim 26 wherein the clinical marker of toxicity is a plasma level of the antifolate compound greater than a predetermined level indicating toxicity at a given time after administration of the compound.

28. Use according to Claim 27 wherein the predetermined plasma level of the antifolate compound indicating toxicity is 1μM at 24 hours after administration of the compound.

29. Use according to any of Claims 25 to 28 for combating toxicity in an individual who has one of more clinical symptoms of toxicity caused by the antifolate compound.

30. Use according to Claim 29 wherein the clinical symptom of toxicity caused by the antifolate compound is selected from anaemia, anorexia, asthenia, dehydration, diarrhoea, fatigue, fever, hepatotoxicity, hyperbilirubinaemia, leukopaenia, mucositis, myelosuppression, nausea, neutropaenia, rash, reversible transaminitis, stomatitis, thrombocytopaenia and vomiting.

31. Use of an antifolate compound of Formula I as defined in Claim 1 or Claim 2 in the preparation of a medicament for combating a disease combatable by said antifolate compound in an individual who is subsequently administered an enzyme that has carboxypeptidase G activity.

32. Use according to any of Claims 25 to 31 for combating toxicity in an individual who is administered a folate pathway rescue agent.

33. Use according to Claim 32 wherein the individual is administered the folate pathway rescue agent prior to the enzyme that has carboxypeptidase G activity.

34. Use according to Claim 32 wherein the individual is administered the folate pathway rescue agent after the enzyme that has carboxypeptidase G activity.

35. Use according to Claim 32 wherein the individual is administered the folate pathway rescue agent and the enzyme that has carboxypeptidase G activity substantially simultaneously.

36. Use of a folate pathway rescue agent in the preparation of a medicament for combating toxicity caused by an antifolate compound of Formula I as defined

in Claim 1 or Claim 2 in an individual who is administered an enzyme that has carboxypeptidase G activity.

37. Use of an enzyme that has carboxypeptidase G activity and a folate pathway rescue agent in the preparation of a medicament for combating toxicity caused by an antifolate compound of Formula I as defined in Claim 1 or Claim 2.

38. Use according to any of Claims 32 to 37 wherein the antifolate compound is an inhibitor of DHFR or GARFT, and the folate pathway rescue agent is leucovorin.

39. Use according to Claim 38 wherein the antifolate compound is LY309887, AG2034, or AG2037.

40. Use according to any of Claims 32 to 37 wherein the antifolate compound of Formula I is an inhibitor of TS, and the folate pathway rescue agent is thymidine.

41. Use according to Claim 40 wherein the antifolate compound of Formula I is Tomudex.

42. Use according to any of Claims 25 to 41 wherein the enzyme that has carboxypeptidase G activity is at a dose of about 50 Units per kg body weight.

43. Use according to any of Claims 25 to 42 for combating toxicity caused by an antifolate compound of Formula I in an individual who is being treated for a disease selected from cancer, RA, MS, psoriasis, extrauterine pregnancy and graft vs. host disease by administration of the antifolate compound.

44. Use of an antifolate compound of Formula I as defined in Claim 1 or Claim 2 in the preparation of a medicament for treating a condition selected from cancer,

RA, MS, psoriasis, extrauterine pregnancy and graft vs. host disease in an individual who is subsequently administered an enzyme that has carboxypeptidase G activity.

45. Use of an enzyme that has carboxypeptidase G activity in the preparation of a medicament for complementing the therapy of a disease selected from cancer, RA, MS, psoriasis, extrauterine pregnancy and graft vs. host disease that is being treated by administration of an antifolate compound of Formula I.

46. Use according to any of Claims 43 to 45 wherein the antifolate compound of Formula I and the cancer to be treated are as defined in any of Claims 21-24.

47. A therapeutic system comprising an antifolate compound of Formula I as defined above in Claim 1 or 2, and an enzyme that has carboxypeptidase G activity.

48. A therapeutic system according to Claim 47 further comprising a folate pathway rescue agent.

49. An *ex vivo* method of cleaving a terminal L-glutamate moiety from a compound of Formula I as defined in Claim 1 or Claim 2, the method comprising contacting the compound with an enzyme that has carboxypeptidase G activity.

50. A method of determining the rate and/or extent of cleavage of a compound of Formula I as defined in Claim 1 or Claim 2 by an enzyme that has carboxypeptidase G activity, the method comprising:

providing the compound of Formula I,

contacting the compound of Formula I with an enzyme that has carboxypeptidase G activity under conditions such that cleavage of the compound can occur, and

monitoring the rate and/or extent of cleavage of the compound of Formula I over time.

51. A method according to Claim 50 wherein the monitoring step comprises monitoring the amount and/or concentration of the compound of Formula I.

52. A method according to Claim 50 or 51 wherein the monitoring step comprises monitoring the amount and/or concentration of one or more break-down products of the compound of Formula I.

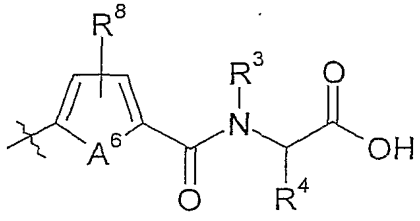
53. A method according to any of Claims 50 to 52 which is performed *ex vivo*.

54. A method according to any of Claims 50 to 52 which is performed *in vivo*.

55. A method according to Claim 54 further comprising determining whether an additional dose of the enzyme that has carboxypeptidase G activity is required in order reduce the amount of the compound of Formula I to a predetermined level.

56. A method according to Claim 54 or 55 further comprising contacting the compound of Formula I with an additional dose of the enzyme that has carboxypeptidase G activity under conditions such that cleavage of the compound can occur.

57. A method of cleaving a compound comprising a structural fragment of Formula VIII,




VIII

wherein

the wavy line indicates the point of attachment of the structural fragment;

$A^6$ represents O or S;

$R^8$ represents H or one or two substituents selected from halo, $C_{1-4}$ alkyl and $C_{1-4}$ alkoxy;

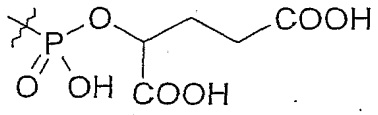
$R^3$ represents H or $C_{1-4}$ alkyl;

$R^4$ represents $-CH_2C(R^{9a})(R^{9b})$-D;

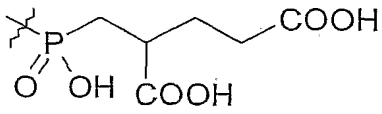
$R^{9a}$ and $R^{9b}$ independently represent H or $C_{1-4}$ alkyl, or $R^{9a}$ and $R^{9b}$ together represent $=C(H)R^{10}$;

$R^{10}$ represents H or $C_{1-4}$ alkyl;

D represents C(O)OH, tetrazol-5-yl, $(CH_2)_{0-1}$-$NHR^{11}$, or, when $R^{9a}$ and $R^{9b}$ together represent $=C(H)R^{10}$, then D may also represent H, or D represents a structural fragment of Formula IIIa or IIIb,




IIIa




IIIb ;

wherein the wavy lines indicate the point of attachment of the structural fragments;

$R^{11}$ represents H or $C(O)R^{12}$;

$R^{12}$ represents H or phenyl substituted by C(O)OH and optionally substituted by one or two further substituents selected from halo, $C_{1-4}$ alkyl and $C_{1-4}$ alkoxy; and

alkyl, alkenyl and alkynyl groups, as well as the alkyl part of alkoxy groups, may be substituted by one or more halo atoms;

or a pharmaceutically acceptable salt and/or solvate thereof,

the method comprising contacting the compound comprising the structural fragment of Formula VIII with an enzyme that has carboxypeptidase G activity.

58. A method according to Claim 57 that is performed *ex vivo*.

59. A method according to Claim 57 that is performed *in vivo*.

60. A method according to Claim 57 wherein the compound comprising the structural fragment of Formula VIII is an antifolate compound.

61. A method according to Claim 60 for combating toxicity caused by the antifolate compound in an individual who has been administered the said antifolate compound in the course of medical treatment, or otherwise, the method comprising administering to the individual an enzyme that has carboxypeptidase G activity.

62. Use of an enzyme that has carboxypeptidase G activity in the preparation of a medicament for combating toxicity caused by an antifolate compound of Formula VIII as defined in Claim 57.

63. A method according to any of Claims 1 to 24 or 49 to 61, or a use according to any of Claims 25 to 46 or 62, or a therapeutic system according to Claim 47 or 48, wherein the enzyme that has carboxypeptidase G activity is carboxypeptidase $G_2$, or a derivative thereof which has carboxypeptidase G activity.